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Insulin-like growth factor binding protein 1 stimulates cell migration and binds to the $\alpha_5\beta_1$ integrin by means of its Arg-Gly-Asp sequence

(Chinese hamster ovary/fibronectin receptor/mutagenesis)

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ABSTRACT **Insulin-like growth factor (IGF)-binding protein 1 (IGFBP-1) contains an Arg-Gly-Asp (RGD) integrin recognition sequence. *In vitro* mutagenesis was used to alter this RGD sequence to Trp-Gly-Asp (WGD). Migration of Chinese hamster ovary (CHO) cells expressing the wild-type protein was more than 3-fold greater in 48 hr compared with cells expressing the WGD mutant form of IGFBP-1. Similarly, wild-type IGFBP-1 added to the media of control CHO cells stimulated migration 2-fold compared with the WGD protein. A synthetic RGD-containing peptide, when added to the medium with wild-type IGFBP-1, blocked the effect of IGFBP-1 on cell migration. The addition of IGF-I to the culture medium had no effect on the migration of cells expressing IGFBP-1 or vector alone. Affinity chromatography of ^{125}I-labeled CHO cell membrane proteins, using IGFBP-1 coupled to agarose, identified the $\alpha_5\beta_1$ integrin (fibronectin receptor) as the only cell surface molecule capable of binding IGFBP-1 in an RGD-dependent manner. Furthermore, wild-type IGFBP-1, but not the WGD mutant form, could be coprecipitated from CHO cells with an antibody directed against the α_5 integrin subunit. These studies demonstrate that IGFBP-1 stimulates CHO cell migration and binds to the $\alpha_5\beta_1$ integrin receptor, both by an RGD-dependent mechanism. The effect of IGFBP-1 on migration is independent of IGF-I and is probably mediated through the $\alpha_5\beta_1$ integrin.**

The insulin-like growth factor (IGF)-binding proteins (IGFBPs) are a family of homologous but distinct proteins that specifically bind IGF-I and IGF-II with high affinity and are ubiquitous in biologic fluids, tissues, and the extracellular matrix of most cell types (1). Currently six IGFBPs have been purified, cloned, and sequenced (2, 3). The roles of these proteins in intercellular transport, extracellular localization, and the modulation of the actions of IGF-I and IGF-II are areas of active study. The major focus of studies published to date has been to examine the effects of the IGFBPs on modifying IGF physiology. However, few direct non-IGF-mediated effects of a purified form of IGFBP on cellular functions have been reported. IGFBP-1 is a phosphorylated protein of approximately 25 kDa that is expressed in greatest amounts during fetal development. It has been reported to potentiate (4) or inhibit (5) IGF actions, depending upon the experimental conditions and degree of IGFBP-1 phosphorylation (6). IGFBP-1 contains an Arg-Gly-Asp (RGD) integrin recognition sequence (7, 8) and has been shown to bind to cell surfaces (9). The specific mechanism by which IGFBP-1 or any of the other IGFBPs bind to cell surfaces has not been previously described.

We undertook these studies after observing that transfected Chinese hamster ovary (CHO) cells expressing human IGFBP-1 appeared to spread and migrate on tissue culture plates more rapidly than CHO cells not expressing IGFBP-1. Cell migration is known in many cell types to involve cellular integrin receptor binding to RGD sequences in extracellular matrix proteins (10). Since IGFBP-1 binds to cell surfaces and contains an RGD sequence, we sought to determine whether the increase in cell migration in cells expressing IGFBP-1 required the presence of this integrin recognition sequence and whether IGFBP-1 bound to a cell surface integrin.

METHODS

Cells and Expression Vectors. The pNUT expression vector is a PUC-18-derived plasmid (11) (provided by Richard Palmiter, University of Washington) containing a dihydrofolate reductase gene (12) driven by a simian virus 40 promoter and a mouse metallothionein promoter (13) driving expression of a human growth hormone gene. An IGFBP-1 expression vector was constructed (6) by removing the growth hormone gene and ligating in its place a cDNA encoding human IGFBP-1. Polymerase chain reaction (PCR) was used to mutate the IGFBP-1 coding sequence at the RGD site by using a mutagenic primer which was complementary to the IGFBP-1 cDNA and which encoded an Arg-to-Trp (R-to-W) substitution at amino acid 221. The wild-type IGFBP-1 cDNA was used as template. The PCR product was completely sequenced and ligated into pNUT downstream from the metallothionein promoter to construct an expression vector for [Trp^{221}]IGFBP-1. A control plasmid was constructed by removing the growth hormone gene from pNUT and religating the remaining plasmid. This control plasmid, the IGFBP-1 construct, and the [Trp^{221}]IGFBP-1 mutant construct were each transfected into CHO-K1 cells (American Type Culture Collection) by calcium phosphate precipitation (14).

Transfected CHO-K1 cells were selected and amplified into stable cell lines with methotrexate (50 μM final concentration). Three cell lines were used: CHOpNUT-A, containing the pNUT expression vector alone; CHOBP1-D6, expressing IGFBP-1; and CHOWGD-A6, expressing the RGD-to-WGD mutant IGFBP-1. Radioimmunoassay (RIA) (15) of the conditioned medium obtained from confluent monolayers showed that the CHOBP1-D6 cells secreted approximately 8 μg of IGFBP-1 per ml in 48 hr. By ligand blot analysis (16) and scanning laser densitometry, the CHOWGD-A6 cells secreted approximately 4 μg of [Trp^{221}]IGFBP-1 per ml in 48 hr as estimated by comparison with wild-type IGFBP-1 standards. To determine IGF mRNA abundance, total cellular RNA was prepared (17) from the transfected CHO cell lines and was subjected to Northern blot analysis (18), using rat

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Abbreviations: IGF, insulin-like growth factor; IGFBP, IGF-binding protein; CHO, Chinese hamster ovary.

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IGF-I (19) and IGF-II (20) probes. To quantify IGF secretion, media (6 ml) conditioned for 24 and 48 hr by the CHOp-NUT-A cells were lyophilized, resuspended in 1 M acetic acid with ^{125}I-labeled IGF-I, and fractionated by a Sephadex column (G-50) to separate hamster IGFBP from the IGF peptides. The fractions containing free ^{125}I-IGF-I were pooled and then analyzed by RIA for IGF-I (21) and IGF-II (22). This pool was also analyzed by using an IGF binding assay (9) to determine the amount of residual IGFBP in these fractions. A 24-kDa IGFBP (presumably hamster IGFBP-4) that was secreted by control CHO cells was purified to homogeneity (23) and was used in the IGF binding assay as a standard.

Cell Wounding and Migration Assay. Cells were plated at 1.2–1.4 × 10^4 cells per cm^2 in α-MEM and 10% dialyzed fetal bovine serum and allowed to grow to confluency over 3 days. Wounding was performed by using a razor blade to scrape cells off the culture plate, leaving a denuded area and a sharp visible demarcation line at the wound edge (24, 25). The wounded monolayers were rinsed twice and incubated in serum-free α-MEM with 0.01% bovine serum albumin with or without other test substances added to the media. The plates were inspected immediately after wounding, and the sections of the wounds (1.0 mm in length) that were to be used for quantitating migration were selected, marked, and numbered. The cells were then incubated for 48 hr, rinsed in phosphate-buffered saline (PBS; Sigma), fixed in 100% methanol, and examined and photographed under phase-contrast microscopy. Cells migrating 125 and 175 μm into the denuded area were counted in sections that were 1.0 mm in length. A calibrated eyepiece grid was used to determine distances. The mean number of cells migrating per 1.0-mm section of wound was calculated by averaging a mean of 10 sections (range 5 to 25) per test substance for each experiment.

Proteins and Peptides. Test substances added to the media during the cell migration assay included human IGFBP-1 and [Trp^{221}]IGFBP-1 [purified to homogeneity from CHO cell conditioned media as previously described (26)], human recombinant IGF-I (Bachem), and three synthetic HPLC-purified peptides: (*i*) the 13 amino acids of the sequence of IGFBP-1 from Gly-216 to Ile-228, containing the Arg-Gly-Asp sequence in residues 221–223 as well as 5 amino acids on each side (a gift from Andreas Sommer, Synergen, Boulder, CO), (*ii*) Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP), and (*iii*) Gly-Arg-Gly-Glu-Ser-Pro (GRGESP) (GRGDSP and GRGESP were gifts from David Klapper, University of North Carolina). The IGF-I-binding affinities of the two forms of IGFBP-1 were determined by a previously described solution binding assay (6).

IGFBP-1 Affinity Chromatography and Anti-Integrin Immunoprecipitation. IGFBP-1 affinity matrix was prepared by linking 1.5 mg of human IGFBP-1 [purified from human amniotic fluid (26)] to 3 ml of carbonyldiimidazole-activated agarose (Reactigel 6X, Pierce), according to the manufacturer's instructions. CHOpNUT cells (≈5 × 10^7 cells) were dispersed with an enzyme-free dispersant (Sigma), surface labeled for 10 min at 22°C with 1 mCi (37 MBq) of carrier-free Na^{125}I (Amersham) and Iodo-Beads (Pierce) according to the manufacturer's instructions, washed, and then lysed at 4°C for 30 min with 100 mM *n*-octyl glucoside in Hepes-buffered saline (150 mM NaCl/40 mM Hepes, pH 7.3/1 mM $CaCl_2$/1 mM $MgSO_4$/1 mM $MnCl_2$/1 mM phenylmethanesulfonyl fluoride/1 mM benzamidine). The detergent-soluble lysate (0.6 ml) was applied to a 3-ml column of the IGFBP-1 affinity matrix, which had been preequilibrated with elution buffer (25 mM *n*-octyl glucoside in Hepes-buffered saline). The column was clamped overnight and then bound material was eluted with elution buffer at 6 ml/hr. GRGDSP and GRGESP peptides were added to the elution buffer as described in the legend to Fig. 4*A*. Fractions (1 ml) were concentrated 25-fold by using a Centricon-30 microconcentrator (Amicon) and analyzed by SDS/7% polyacrylamide gel electrophoresis with autoradiography of the dried gels. Fractions containing labeled cell surface proteins that were eluted from the column with GRGDSP peptide were immunoprecipitated by using polyclonal antibodies raised against synthetic peptides containing sequences from the highly conserved cytoplasmic tail regions of the human α_2, α_3, α_5, α_v, and β_1 integrin subunits (27). These antibodies recognize integrins from many species, including hamster, and were the generous gift of Guido Tarone (University of Torino, Italy). Coimmunoprecipitation of IGFBP-1 (vs. [Trp^{221}]IGFBP-1) with $\alpha_5\beta_1$ integrin was performed as described in the legend for Fig. 5.

RESULTS

Migration of CHO Cells Is Stimulated by IGFBP-1 Through an RGD-Dependent Mechanism. Stable methotrexate-resistant cell lines of transfected CHO cells were established that expressed and secreted human IGFBP-1 or the [Trp^{221}]IGFBP-1 mutant in which the Arg-Gly-Asp sequence was changed to Trp-Gly-Asp (WGD). Monolayers of these cells were wounded, and the typical appearance of the migrating cells is shown in Fig. 1. The control cells (transfected with vector alone) and the cells expressing [Trp^{221}]IGFBP-1 demonstrated minimal movement into the denuded area. In contrast, the cells expressing wild-type IGFBP-1 migrated in large numbers well into the denuded area, with many cells moving more than 125 or 175 μm past the wound edge (dashed arrows). Similarly, control cells incubated with wild-type IGFBP-1 added to the serum-free medium migrated in large numbers into the denuded area. However, treatment of control cells with [Trp^{221}]IGFBP-1 had no apparent effect on cell migration or morphology (not shown in Fig. 1).

We quantitated these observations by counting the number of cells that had migrated more than 125 and 175 μm past the wounded edge at the end of the 48-hr migration period. Cells migrating less than 125 μm past the wound edge could have entered the denuded area solely as a result of division of the cells at the edge and were therefore not included in the analysis. However, even if the cells migrating less than 125 μm were included, the results obtained were not qualitatively different. Fig. 2 demonstrates that cells expressing wild-type

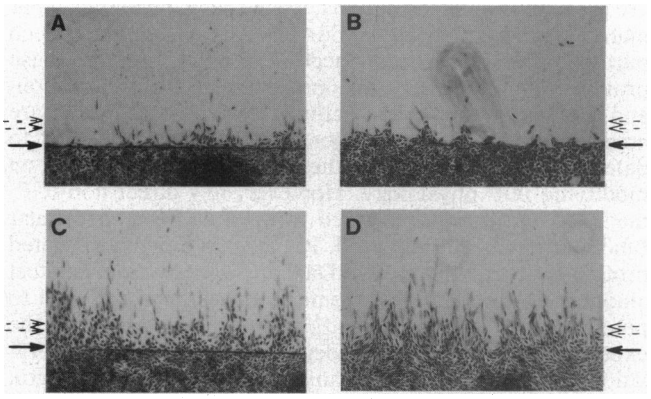

FIG. 1. Morphology of CHO cells after wounding and migration. CHO cells transfected with the pNUT vector alone (*A*; control cells), the RGD-to-WGD mutant [Trp^{221}]IGFBP-1 (*B*), and wild-type IGFBP-1 (*D*) were grown to confluence, wounded with a razor blade, and allowed to migrate into the denuded area for 48 hr in serum-free medium. In *C* control cells were exposed to wild-type IGFBP-1 (1.0 μg/ml) that was added to the medium during the 48-hr incubation. The solid arrows mark the wound edges, and the dashed arrows mark 125 and 175 μm of migration. Migration was quantitated by counting the number of cells migrating beyond these distances.

IGFBP-1 migrated >125 and >175 μm in 3-fold greater numbers than control cells, while the migration of cells expressing [Trp^{221}]IGFBP-1 was indistinguishable from control. The clone of cells used in these experiments (CHOBP1-D6) secreted approximately 2-fold more wild-type IGFBP-1 compared with the amount of [Trp^{221}]IGFBP-1 secreted from CHOWGD-A6 cells. However, a separate clone (CHOBP1-E4), which secreted an amount of wild-type IGFBP-1 equivalent to the amount of mutant protein secreted by the CHOWGD-A6 cells, also migrated in 3-fold greater numbers than the CHOWGD-A6 cells.

Since constitutive expression of IGFBP-1 increased cell migration, we determined whether treatment with exogenous IGFBP-1 would stimulate migration of the control cells. Exogenous IGFBP-1, when added to the medium of control cells, caused a concentration-dependent increase in stimulation of migration, with a response that was significant at 100 ng/ml and maximal at 1 μg/ml and above (not shown). While the cells expressing IGFBP-1 demonstrated a 3-fold increase in migration compared with control cells in serum-free medium, treatment of control cells with pure IGFBP-1 (1 μg/ml) increased their migration 2-fold (Fig. 3). In contrast to the wild-type protein, treatment of control cells with [Trp^{221}]IGFBP-1 at concentrations of 1 μg/ml (Fig. 3) or up to 10 μg/ml (data not shown) had no effect. The loss of effect on cell migration by [Trp^{221}]IGFBP-1 was not due to difference in affinity for IGF-I, since the affinities for IGF-I of the two forms of IGFBP-1 (measured by Scatchard analysis) were found to be indistinguishable ($K_a = 1.0 \times 10^9\ M^{-1}$ at pH = 7.0). Importantly, the effect of wild-type IGFBP-1 could be completely blocked by the simultaneous addition of a synthetic peptide containing the RGD sequence, while the addition of the same concentration of a control peptide (GRGESP) had no effect (Fig. 3). Addition of the RGD peptide to control cells in the absence of IGFBP-1 similarly had no effect (not shown). When cells expressing [Trp^{221}]IGFBP-1 were treated with wild-type IGFBP-1 at 1 μg/ml, they responded with a 1.8-fold increase in migration (not shown). This response was indistinguishable from the response of control cells treated with IGFBP-1 (Fig. 3).

Migration Is Independent of IGF-I. CHO cells have IGF-I receptors (demonstrated by affinity cross-linking) and demonstrate a proliferative response to IGF-I at concentrations between 10 and 100 ng/ml (not shown). In contrast, when migration studies were performed in the presence of the same concentrations of IGF-I no effect on migration was detected in any of the three CHO cell lines studied (not shown). We examined the possibility that CHO cells secreted IGF-I or

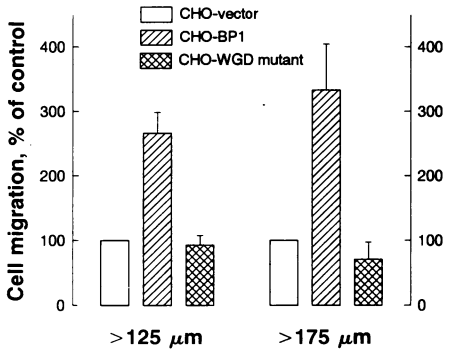

FIG. 2. Migration of three transfected CHO cell lines in serum-free medium. The cells migrating >125 μm and >175 μm into the denuded area per 1.0-mm wound edge were counted and the mean (±SEM) is expressed as a percentage of control cell migration. The control CHO cells were transfected with the pNUT vector alone, and the cells expressing IGFBP-1 and the WGD mutant IGFBP-1 ([Trp^{221}]IGFBP-1) were transfected as described in *Methods*.

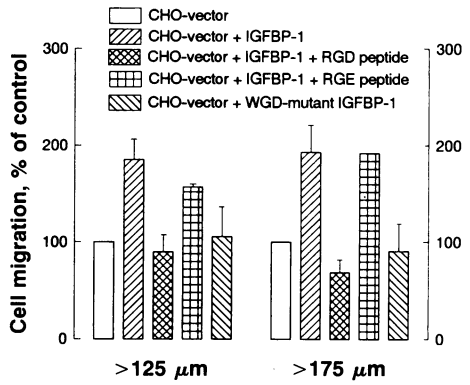

FIG. 3. Blocking of the response of CHO cells to IGFBP-1 by an RGD-containing peptide and by mutation of the RGD sequence of IGFBP-1. Control CHO cells were wounded and allowed to migrate in the presence of either IGFBP-1 or the RGD-to-WGD mutant [Trp^{221}]IGFBP-1 at 1 μg/ml. In some plates both IGFBP-1 and either a synthetic 13-amino acid peptide containing RGD or RGE control peptide at 30 μM were added simultaneously. Migration is expressed as a percent of migration by control cells in the absence of IGFBP-1. Peptides were dissolved in dimethyl sulfoxide (final assay concentration, 0.25%), and therefore control cells for the peptide experiments were treated with 0.25% dimethyl sulfoxide (which did not affect their migration).

IGF-II; no IGF-I or IGF-II mRNA could be detected in the cell lysates by Northern analysis, and no IGF-I or IGF-II peptide could be detected in CHO conditioned media after acid gel filtration. While there was some displacement of ^{125}I-IGF in the IGF-I and IGF-II RIAs, analysis of the gel filtration fractions revealed that they contained a small amount (<2% of the total) of the 24-kDa form of hamster IGFBP. This amount of purified 24-kDa hamster IGFBP, when added to the IGF-I and IGF-II RIAs, resulted in approximately the same ligand displacement. Therefore all of the apparent IGF-I and IGF-II in this pool of fractions was due to interference by the 24-kDa IGFBP in the RIA.

IGFBP-1 Binds to the $\alpha_5\beta_1$ Integrin by an RGD-Dependent Mechanism. To determine whether a cell surface integrin was capable of recognizing IGFBP-1 in an RGD-dependent fashion, we followed procedures previously used to identify fibronectin and vitronectin receptors (28). ^{125}I-labeled surface proteins were subjected to affinity chromatography using immobilized IGFBP-1. Labeled proteins of approximately 150 and 110 kDa (unreduced) were specifically eluted from the column by the synthetic peptide GRGDSP but not by the control peptide GRGESP (Fig. 4*A*). When these proteins were reduced, their apparent molecular masses changed to 135 and 130 kDa (Fig. 4*B*). The affinity-purified ^{125}I-labeled proteins were immunoprecipitated with anti-integrin antibodies (Fig. 4*C*). The antibodies directed against the α_5 and β_1 integrin subunits each precipitated both bands, while the antibodies against the α_2, α_3, and α_v subunits precipitated neither, indicating that the cell surface complex which bound to immobilized IGFBP-1 was the heterodimeric $\alpha_5\beta_1$ integrin receptor. Furthermore, the anti-α_5 integrin antibody coprecipitated soluble IGFBP-1 (Fig. 5, lane 2) but not [Trp^{221}]IGFBP-1 (lane 3) from cell lysates, while nonimmune serum did not (lanes 1 and 4), providing definitive proof that IGFBP-1 associates with the $\alpha_5\beta_1$ integrin in an RGD-dependent fashion.

DISCUSSION

Both the expression of IGFBP-1 and the treatment of non-expressing cells with exogenous IGFBP-1 resulted in significant increases in migration of CHO cells. This effect was dependent on the presence in IGFBP-1 of an intact RGD

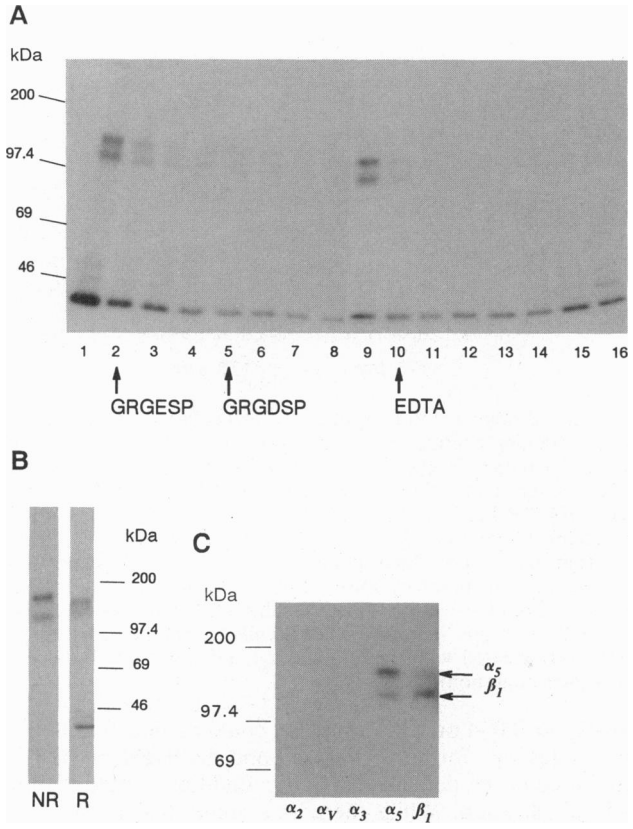

FIG. 4. IGFBP-1 affinity chromatography. (*A*) CHOpNUT cells were surface-labeled with ^{125}I, the detergent cell lysate was applied to a 3-ml affinity column of immobilized IGFBP-1, and retained proteins were eluted. The first 8 ml of eluate was discarded, and subsequent 1-ml fractions were collected and concentrated 25-fold, and 20 μl of the concentrate was subjected to SDS/7% PAGE under nonreducing conditions. The autoradiograph of the dried gels containing fractions 1–16 is shown. The arrows indicate the times at which the GRGESP and GRGDSP peptides (1 mg/ml for 3 ml), or EDTA (10 mM for 5 ml) were first added to the elution buffer. Two bands, of 150 and 110 kDa, were retained by the column and were still equilibrating with the buffer in fractions 1–4. While no effect of the addition of the GRGESP control peptide was observed, the addition of the GRGDSP peptide to the elution buffer caused the 150- and 110-kDa bands to elute in fractions 9 and 10. The addition of EDTA, which causes the 150/110-kDa proteins to be completely eluted from the column (not shown), resulted in no further elution of the 150/110-kDa proteins, but it did cause an unidentified 46-kDa band to be eluted in fractions 15 and 16. (*B*) The ^{125}I-labeled proteins eluting in fraction 9 have an apparent molecular mass of 150 and 110 kDa when not reduced (NR) and apparent masses of 135 and 130 kDa after reduction with dithiothreitol (R). (*C*) Pooled IGFBP-1 affinity fractions containing the 150/110-kDa ^{125}I-labeled proteins were immunoprecipitated with antibodies directed against the cytoplasmic domains of the α_2, α_v, α_3, α_5, and β_1 integrin receptor subunits. The α_5 and β_1 antibodies successfully precipitated both bands in the 150/110-kDa complex (arrows) indicating that the 150-kDa band is the α_5 integrin subunit and the 110-kDa band is the β_1 subunit.

sequence, since both the mutation of this sequence to WGD ([Trp^{221}]IGFBP-1) and the addition of excess RGD peptide abrogated the effect. Significantly, the effect on migration of IGFBP-1 in this system was independent of IGF-I, since (*i*) the WGD mutation did not affect binding affinity for IGF-I, (*ii*) addition of IGF-I to the medium had no effect on migration of control cells or of cells expressing either IGFBP-1 or [Trp^{221}]IGFBP-1, and (*iii*) no endogenous IGF-I or IGF-II or their mRNAs could be detected. The observation of an IGF-independent effect of IGFBP-1 and the demonstration of a functional significance of its RGD sequence are both, as far as we know, novel findings.

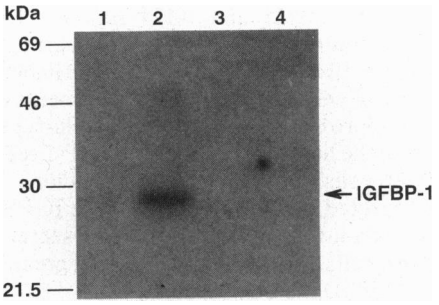

FIG. 5. Coprecipitation of IGFBP-1 with $\alpha_5\beta_1$ integrin. Confluent CHOBP1-D6 cells (lanes 1 and 2) and CHOWGD-A6 cells (lanes 3 and 4) in 10-cm dishes were incubated in serum-free medium for 24 hr then lysed in 0.5 ml of 25 mM *n*-octyl glucoside in Hepes-buffered saline for 60 min. The detergent-soluble lysates (150 μl) were incubated overnight at 4°C with 1 μl of rabbit polyclonal anti-α_5 integrin antiserum (lanes 2 and 3) or nonimmune rabbit serum (lanes 1 and 4) and IGFBP-1 (lanes 1 and 2) or [Trp^{221}]IGFBP-1 (lanes 3 and 4) at 1 μg/ml. The IGFBP concentration was chosen to approximate that present in the conditioned media of these cells. The resulting ligand–integrin–antibody complexes were precipitated with staphylococcal protein A-Sepharose (Sigma) and washed three times in the lysis buffer, and the IGFBP-1 (or [Trp^{221}]IGFBP-1) in the precipitates was determined by SDS/12.5% PAGE and ligand blotting using ^{125}I-IGF-I (16). The autoradiograph of the ligand blot is shown. The arrow indicates the location of the IGFBP-1, which was precipitated by anti-α_5 (lane 2), while the [Trp^{221}]IGFBP-1 did not coprecipitate (lane 3). The nonimmune control serum (lanes 1 and 4) did not precipitate any detectable IGFBP.

The migration results strongly suggested that IGFBP-1 had its effect on migration by directly interacting with cell surface integrin receptors. In addition to the classic fibronectin receptor, the $\alpha_5\beta_1$ integrin, six other integrins have been reported to recognize the RGD sequence: the $\alpha_3\beta_1$, $\alpha_v\beta_1$, $\alpha_{IIb}\beta_3$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_v\beta_6$ integrins (8, 29). We tested the hypothesis that IGFBP-1 was a ligand for one or more of these integrins by applying a surface-labeled cell lysate to an affinity column of immobilized IGFBP-1, and we determined that the $\alpha_5\beta_1$ integrin heterodimer was the only cell surface protein that bound to the column in an RGD-dependent manner. Confirmation that soluble IGFBP-1 associates with the $\alpha_5\beta_1$ integrin was provided by the specific coprecipitation of IGFBP-1 with the α_5 integrin subunit directly from cell lysates. Significantly, the WGD mutant form of IGFBP-1 did not coprecipitate with the α_5 integrin, confirming the RGD dependence of binding. These experiments identify a specific cell surface molecule that recognizes an IGFBP, and they establish IGFBP-1 as the second known ligand, after fibronectin, for the $\alpha_5\beta_1$ integrin.

Since both the effect of IGFBP-1 on cell migration and its ability to associate with the $\alpha_5\beta_1$ integrin depend on an intact RGD sequence, it is likely that stimulation of migration in our system was mediated by binding of IGFBP-1 to the $\alpha_5\beta_1$ integrin receptor. The mechanism by which the interaction between IGFBP-1 and the integrin results in increased migration is unknown. Cell adhesion molecules such as integrins and their ligands clearly have effects on cell migration. In CHO cells fibronectin stimulates migration, and the presence of the $\alpha_5\beta_1$ integrin is required for cell migration to occur (30), but overexpression of the same integrin in CHO cells results in decreased migration (31). An explanation for these observations that has been proposed is that, in the absence of this integrin, adhesion to the substratum is insufficient to allow cell traction, while excessive adhesion resulting from overexpression of the integrin results in decreased mobility (29). However, the adhesion of control CHO cells to wells coated with IGFBP-1 is not different than adhesion to bovine serum albumin-coated control wells (R. Juliano and J.I.J.,

unpublished observations), suggesting that the stimulation of migration by IGFBP-1 is not due to a direct effect of IGFBP-1 on CHO cell adhesiveness. This would suggest that binding of IGFBP-1 to the integrin receptor results in an intracellular signaling event, which causes an increase in cell motility. Such signaling functions have been suggested for integrins, and intracellular sequelae of integrin stimulation include activation of protein tyrosine kinases (32) such as $p125^{FAK}$ (33), cytoplasmic alkalinization (34), and induction of the AP-1 transcription factor (35). Transmission of a putative intracellular signal resulting from IGFBP-1 binding to $\alpha_5\beta_1$ integrin would require not only an intact RGD sequence but also some other region of IGFBP-1, since the 13-amino acid RGD peptide by itself did not stimulate migration. In this regard, IGFBP-1 is similar to fibronectin, whose ability to activate $\alpha_5\beta_1$ integrin receptors depends not only on the presence of its RGD sequence but also on a region of the protein approximately 170 residues removed from the RGD site (36). Moreover, the effects of fibronectin on cell migration are also blocked by short RGD-containing peptides (37). Finally, the existence of an RGD-containing integrin ligand which is not adhesive but stimulates migration is not without precedent: tenascin is a migration-stimulating molecule that has anti-adhesive properties (38) yet binds to cells in an RGD-dependent fashion (39).

This effect of IGFBP-1 on cell migration may have important physiologic consequences. IGFBP-1 is highly expressed in multiple fetal tissues (40), in decidualized endometrium (41), and in regenerating liver (42). Concentrations of IGFBP-1 comparable to or greater than those used in these studies are likely to be present in the pericellular environment of these tissues, where the $\alpha_5\beta_1$ integrin is also expressed. These are tissues in which coordinated cell migration is known to occur. Previously, biologic effects of IGFBP-1 in these and other systems had been believed to be limited to the modulation of the effects of the IGFs. However, to properly interpret such results it will be necessary to consider direct effects of IGFBP-1. These findings raise the possibility that the other IGFBPs (in particular IGFBP-2, which also contains the RGD sequence) may also bind to integrins or other cell surface receptors.

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